

Y4345 SEQUENCE LISTING.txt  
SEQUENCE LISTING

<110> University of Wales, Bangor  
Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

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aaacttaccc cggaacaggc cgagcagatc aaaacgctac tgcaatacag cccatccagc 120  
accaactccc agccgtggca ttttattggt gccagcacgg aagaaggtaa agcgcgtggt 180  
gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgcac 240  
gtcgtgggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttggtgac 300  
caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt 360  
cgcaagttct tcgctgatat gcaccgtaaa gatctgcatg atgatgcaga gtggatggca 420  
aaacagggtt atctcaacgt cggttaacttc ctgctcggcg tggcggctct ggggtctggac 480  
gcggtaccca tcgaagggtt tgacgccgcc atcctcgatg cagaatttgg tctgaaagag 540  
aaaggctaca ccagtctggt ggttgttccg gtaggtcatc acagcgttga agattttaac 600  
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<210> 2

<211> 826

<212> DNA

<213> Pseudomonas putida JLR11

<400> 2

Y4345 SEQUENCE LISTING.txt

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agcgatcttc ctgtggatga gcagatgctg agctgggcga tcgcggcggc ccagtcagcc	180
tcgacttcct cgaacctgca agcttggagc gtgctcgccg tgcgggatcg cgagcgtctc	240
gcgaggcttg cccgactgtc cggtaaccag cgccatgtcg agcaggcacc gctgttcctg	300
gtctggctcg tggactggtc acgcctacgc cgactagcca gaacccttca ggcaccgact	360
gcaggtatcg actatttaga aagctacacc gtcggtgttg tagatgcagc tctggccgct	420
cagaacgccg cactagcttt cgaggcccaa ggactgggaa tcgtttacat cggcggaatg	480
cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac tttcgctgta	540
tttggcatgt gcgtcggta tcccgatccg gcacagccc ccgagatcaa gccacgcctg	600
gcgcaatcag tggtgcttca ccgtgagcgc tatgaggcca ccgaggcaga ggcggtttca	660
gttgctgcct atgaccgaag gatgagcgac ttccaacatc gtcaacaacg cgaaaaccgt	720
tcctggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cggaagacac	780
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<210> 3

<211> 1066

<212> DNA

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc\_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc\_feature

## Y4345 SEQUENCE LISTING.txt

&lt;222&gt; (160)..(177)

&lt;223&gt; His tags

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (268)..(285)

&lt;223&gt; primer

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (996)..(1010)

&lt;223&gt; primer

&lt;400&gt; 3

taatacgact cactataggg gaattgtgag cggataacaa ttcccctcta gaaataattt 60

tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat 114  
 Met Gly Ser Ser His His His His His  
 1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162  
 His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
 10 15 20 25

ggc gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210  
 Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp  
 30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258  
 Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala  
 45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306  
 Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu  
 60 65 70

caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt 354  
 Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val  
 75 80 85

gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt 402  
 Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly  
 90 95 100 105

aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg 450  
 Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val  
 110 115 120

gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt 498  
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Val	Phe	Cys	Ala	Lys	Thr	Ala	Met	Asp	Asp	Val	Trp	Leu	Lys	Leu	Val	
			125					130					135			
gtt	gac	cag	gaa	gat	gcc	gat	ggc	cgc	ttt	gcc	acg	ccg	gaa	gcg	aaa	546
Val	Asp	Gln	Glu	Asp	Ala	Asp	Gly	Arg	Phe	Ala	Thr	Pro	Glu	Ala	Lys	
		140					145					150				
gcc	gcg	aac	gat	aaa	ggt	cgc	aag	ttc	ttc	gct	gat	atg	cac	cgt	aaa	594
Ala	Ala	Asn	Asp	Lys	Gly	Arg	Lys	Phe	Phe	Ala	Asp	Met	His	Arg	Lys	
	155					160					165					
gat	ctg	cat	gat	gat	gca	gag	tgg	atg	gca	aaa	cag	ggt	tat	ctc	aac	642
Asp	Leu	His	Asp	Asp	Ala	Glu	Trp	Met	Ala	Lys	Gln	Val	Tyr	Leu	Asn	
170					175					180					185	
gtc	ggt	aac	ttc	ctg	ctc	ggc	gtg	gcg	gct	ctg	ggt	ctg	gac	gcg	gta	690
Val	Gly	Asn	Phe	Leu	Leu	Gly	Val	Ala	Ala	Leu	Gly	Leu	Asp	Ala	Val	
				190					195					200		
ccc	atc	gaa	ggt	ttt	gac	gcc	gcc	atc	ctc	gat	gca	gaa	ttt	ggt	ctg	738
Pro	Ile	Glu	Gly	Phe	Asp	Ala	Ala	Ile	Leu	Asp	Ala	Glu	Phe	Gly	Leu	
			205					210					215			
aaa	gag	aaa	ggc	tac	acc	agt	ctg	gtg	gtt	gtt	ccg	gta	ggt	cat	cac	786
Lys	Glu	Lys	Gly	Tyr	Thr	Ser	Leu	Val	Val	Val	Pro	Val	Gly	His	His	
		220					225					230				
agc	gtt	gaa	gat	ttt	aac	gct	acg	ctg	ccg	aaa	tct	cgt	ctg	ccg	caa	834
Ser	Val	Glu	Asp	Phe	Asn	Ala	Thr	Leu	Pro	Lys	Ser	Arg	Leu	Pro	Gln	
	235					240					245					
aac	atc	acc	tta	acc	gaa	gtg	taa	ttctctcttg	ccgggcatct	gccccgctat						888
Asn	Ile	Thr	Leu	Thr	Glu	Val										
250					255											
ttcctctcag	attctcctga	tttgcataac	cctgtttcag	caagcttcgt	catcataggc											948
tgctgttgaa	gcttgcggcc	gcactcgagc	accaccacca	ccaccactga	gatccggctg											1008
ctaacaaagc	ccgaaaggaa	gctgagttgg	ctgctgccac	cgctgagcaa	taactagc											1066

<210> 4

<211> 256

<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMK2

<220>

<221> misc\_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc\_feature

Y4345 SEQUENCE LISTING.txt

<222> (160)..(177)

<223> His tags

<220>

<221> misc\_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc\_feature

<222> (996)..(1010)

<223> primer

<400> 4

Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro  
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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg  
20 25 30

Gly Ser Cys Cys Cys Cys Cys Cys Asp Ile Ile Ser Val Ala Leu Lys  
35 40 45

Arg His Ser Thr Lys Ala Phe Asp Ala Ser Lys Lys Leu Thr Pro Glu  
50 55 60

Gln Ala Glu Gln Ile Lys Thr Leu Leu Gln Tyr Ser Pro Ser Ser Thr  
65 70 75 80

Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys  
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg  
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala  
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp  
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg  
145 150 155 160

Y4345 SEQUENCE LISTING.txt

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu  
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly  
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala  
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser  
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala  
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Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val  
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<210> 5

<211> 1221

<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

<221> CDS

<222> (88)..(1029)

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<222> (190)..(225)

<223> primer

<220>

<221> misc\_feature

<222> (190)..(207)

<223> cys tag

Y4345 SEQUENCE LISTING.txt

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<221> misc\_feature

<222> (936)..(956)

<223> primer

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat 114  
Met Gly Ser Ser His His His His His  
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162  
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210  
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser  
30 35 40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258  
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro  
45 50 55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306  
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His  
60 65 70

cga tca gta cgt gcc tac agc gat ctt cct gtg gat gag cag atg ctg 354  
Arg Ser Val Arg Ala Tyr Ser Asp Leu Pro Val Asp Glu Gln Met Leu  
75 80 85

agc tgg gcg atc gcg gcg gcc cag tca gcc tcg act tcc tcg aac ctg 402  
Ser Trp Ala Ile Ala Ala Ala Gln Ser Ala Ser Thr Ser Ser Asn Leu  
90 95 100 105

caa gct tgg agc gtg ctc gcc gtg cgg gat cgc gag cgt ctc gcg agg 450  
Gln Ala Trp Ser Val Leu Ala Val Arg Asp Arg Glu Arg Leu Ala Arg  
110 115 120

ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg 498  
Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu  
125 130 135

ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga 546  
Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg  
140 145 150

acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc 594  
Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr  
155 160 165

gtc ggt gtt gta gat gca gct ctg gcc gct cag aac gcc gca cta gct 642  
Val Gly Val Val Asp Ala Ala Leu Ala Ala Gln Asn Ala Ala Leu Ala  
170 175 180 185

Y4345 SEQUENCE LISTING.txt

ttc gag gcc caa gga ctg gga atc gtt tac atc ggc gga atg cgc aac	690
Phe Glu Ala Gln Gly Leu Gly Ile Val Tyr Ile Gly Gly Met Arg Asn	
190 195 200	
cac ccg gaa gcg atg tcc gag gag ctt ggc ctg cca aac gac act ttc	738
His Pro Glu Ala Met Ser Glu Glu Leu Gly Leu Pro Asn Asp Thr Phe	
205 210 215	
gct gta ttt ggc atg tgc gtc ggt cat ccc gat ccg gca cag ccc gcc	786
Ala Val Phe Gly Met Cys Val Gly His Pro Asp Pro Ala Gln Pro Ala	
220 225 230	
gag atc aag cca cgc ctg gcg caa tca gtg gtg ctt cac cgt gag cgc	834
Glu Ile Lys Pro Arg Leu Ala Gln Ser Val Val Leu His Arg Glu Arg	
235 240 245	
tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga	882
Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg	
250 255 260 265	
agg atg agc gac ttc caa cat cgt caa caa cgc gaa aac cgt tcc tgg	930
Arg Met Ser Asp Phe Gln His Arg Gln Gln Arg Glu Asn Arg Ser Trp	
270 275 280	
tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga	978
Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly	
285 290 295	
aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc	1026
Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg	
300 305 310	
tga gatagtgaga tatcccatgc ctattccgc cgccctgaac cggagcacta	1079
atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc	1139
accaccacca ctgagatccg gctgctaaca aagcccgaag ggaagctgag ttggctgctg	1199
ccaccgctga gcaataacta gc	1221

<210> 6

<211> 313

<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

<221> misc\_feature

<222> (190)..(225)

<223> primer

<220>

<221> misc\_feature



## Y4345 SEQUENCE LISTING.txt

&lt;222&gt; (190)..(207)

&lt;223&gt; cys tag

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (936)..(956)

&lt;223&gt; primer

&lt;400&gt; 6

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro  
 1 5 10 15

Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg  
 20 25 30

Gly Ser Cys Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys  
 35 40 45

Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp  
 50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser  
 65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala  
 85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala  
 100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn  
 115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp  
 130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala  
 145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala  
 165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly  
 180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu  
 Page 9

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val  
210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala  
225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu  
245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His  
260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg  
275 280 285

Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala  
290 295 300

Leu Asn Thr Leu Gly Phe Gly Leu Arg  
305 310

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<211> 24

<212> DNA

<213> Escherichia coli

<400> 7

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<210> 8

<211> 27

<212> DNA

<213> Escherichia coli

<400> 8

cgatcatcata ggctgctgtt gaagctt

27

<210> 9

<211> 42

Y4345 SEQUENCE LISTING.txt

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

<400> 9

ggatcctggtt gctgttgctg ttgcgatatc atttctgtcg cc

42

1/11